Biodistribution in rats

Three or four rats per group were used for each biodistribution study. With the rats under ether anesthesia, 0.2 ml of a saline solution containing 50–100 mCi of radioactive tracer was injected into the femoral vein. The rats were sacrificed at the indicated time by cardiac excision while under ether anesthesia. Organs of interest were removed and weighed, and the radioactivity was counted using a Packard automatic gamma counter (Model 5000). The percentage dose per organ was decay corrected and calculated by a comparison of the tissue counts to counts of 1% of the initial dose (100 times diluted aliquots of the injected material) measured at the same time.
Regional brain distribution in rats was measured after an i.v. injection of the radioactive tracer. Samples from different brain regions (cortex, striatum, hippocampus, and cerebellum) were dissected, weighed, and counted. The percentage dose/g of each sample was calculated by comparing sample counts with the counts of the diluted initial dose described above. The ratio of specific uptake in each region was obtained by dividing the difference of each region from the cerebellum (percentage dose/gram) by that of the cerebellum, which contains no dopamine transporters and is used as a background region.
In vivo competitive binding of various compounds in the regional uptake of [99mTc]TRODAT-1 was investigated by pretreating the male animals with b-CIT (or RTI-55, 1 mg/kg, i.v., 5 min prior), or haloperidol (1 mg/kg, i.v., 5 min prior), followed by injection of [99mTc]TRODAT-1. Experiments were performed using groups of three or four animals per study. Similar regional brain distributions were determined as described above.

Blood and brain metabolism

Sixty minutes after an i.v. injection of 400–500 mCi of [99mTc]TRODAT-1 into four male rats, the striatial tissues were dissected out individually and the plasma samples were separated from the red blood cells using centrifugation (3000 g×10 min).
The striatal tissues were then homogenized in 1.5 ml of 0.5 M sodium phosphate buffer (pH 8.0). Both plasma samples (after adjusting the pH to 8.0 with phosphate buffer) and striatial homogenates were extracted with ethyl acetate (3×1.5 ml) in the presence of the Re-TRODAT-1 complex (80 mg). The ethyl acetate layers were evaporated to near dryness and the purity of [99mTc]TRODAT-1 in the condensed extracts was analyzed by HPLC, as discussed above, or by thin layer chromatography (TLC) with Whatman silica gel plates (PESIL G/UV), and a solvent system of ethyl acetate:methanol:triethylamine=80:10:10, Rf=0.72, for [99mTc]TRODAT-1. To avoid the adhesion of [99mTc]TRODAT-1 to the TLC plate, a small amount of Re-TRODAT-1 (10 mg/10 ml) was needed. Controls were determined by adding 10–20 mCi of [99mTc]TRODAT-1 to the striatal tissues or plasmas followed by the same procedures used for the experimental samples to determine the extraction efficiency.
Plasma stability of [99mTc]TRODAT-1 was evaluated by incubating [99mTc]TRODAT-1 with the rat plasma samples at 37° C for 30 min. Following the incubation, the samples were extracted with ethyl acetate after adjusting the pH to 8.0 in the presence of a small amount of Re-TRODAT-1 (80 mg). The purity of [99mTc]TRODAT-1 in the extracted organic layers was determined by TLC as described above. Stability of [99mTc]TRODAT-1 in saline was evaluated under similar conditions.

Ex vivo autoradiography of [99mTc]TRODAT-1 in rat brain

Rats under ether anesthesia were injected intravenously with 0.4 ml of a saline solution containing 8–20 mCi of [99mTc]TRODAT-
1. At 60 min post i.v. injection, the animals were sacrificed by cardiac excision while under ether anesthesia. The brains were rapidly removed, placed in O.C.T. embedding medium (Miles Laboratory, Diagnostics Division, Elkhart, Ind., USA) and frozen with powdered dry ice. After reaching equilibrium at -15° C, consecutive 20-mm coronal sections were cut on a cryostat microtome (Hacker Instruments, Fairfield, NJ, USA), thaw-mounted on gelatin-coated microscope slides, and air dried at room temperature.
The slides containing the brain sections were exposed to DuPont X-ray film in an autoradiographic cassette for 24 h. The exposed film was developed by a Kodak automatic film processor. The optical densities were determined with an image analysis system developed by NIH (Image 1.47). The blocking study was carried out by pretreating the rat with CFT (5 mg/kg, i.v.) 5 min prior to tracer injection. The brain sections of the pretreated rat were processed following the same procedures described above for the normal untreated rat.

Table 1. Comparison of organ distribution and brain regional uptake of [99mTc]TRODAT-1 in male and female rats at 60 min postinjection (percentage dose/organ, average of three rats±SD)

Organ	Male	Female
Blood	1.43±0.08	3.06±0.29
Heart	0.20±0.03	0.26±0.01
Muscle	8.67±2.62	9.18±2.64
Lung	1.28±0.53	2.13±0.63
Kidney	1.90±0.32	2.06±0.34
Spleen	0.41±0.04	0.62±0.17
Liver	23.34±0.98	19.40±2.19
Skin	4.03±0.47	5.10±0.22
Brain	0.11±0.002	0.15±0.01
Regional distribution (percentage dose/g)		
Cerebellum (CB)	0.046±0.004	0.063±0.013
Striatum (ST)	0.127±0.012	0.159±0.014
Hippocampus (HP)	0.071±0.004	0.092±0.010
Cortex (CX)	0.067±0.005	0.090±0.017
ST-CB/CB	1.76	1.52